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Immunohistochemical investigation of vascular endothelial growth factor during the healing of gastric ulcer in female rats: experimental study

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ABSTRACT

The current study attempts to characterize the effects of esomeprazole, curcumin, chitosan, as well as of a mixture of curcumin and chitosan on the expression of the vascular endothelial growth factor (VEGF) in gastric ulcers induced by ethanol in female rats. This study included 30 rats; their average weight ranged between 195.6 and 198.6 g, and the rats were separated into two control groups (negative and positive control groups) and four treatment groups (n=5 rats per group). All groups were treated for 5 days after last administration of distilled water or ethanol. The immunohistochemical staining for VEGF in the cells of gastric mucosa revealed a positive staining in both the control groups and all treated groups. The H-score for VEGF was found to exhibit no significant change ($p>0.05$) in any of the assessed groups.

1. Introduction

Increasing intragastric pH and reducing daily gastric secretion output are two of the most effective ways to decrease gastric acid secretion. Proton pump inhibitors (PPIs) act by inhibiting the stomach H^+,K^+-ATPase (proton pump)[1]. Clinically available PPIs include pantoprazole, rabeprazole, lansoprazole, omeprazole, and esomeprazole. In addition to these PPIs, tenatoprazole has a 5- to 7-fold longer elimination half-life than the aforementioned PPIs[2]. Esomeprazole, the S-isomer of omeprazole (which is a racemic mixture of S- and R- optical isomers), is the first PPIs to be produced as a single optical isomer and has a good pharmacokinetic profile[3].

Curcumin has a long history of

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use in the traditional medicine of India, Iran, and China, and it has been used for the treatment of many diseases (including liver diseases, diabetes, rheumatoid diseases, cancers, infectious diseases, as well as atherosclerosis)[4]. On the other hand, chitosan is regarded as a natural and most promising bio-polymer; it has been included in the GRAS (Generally Recognized as Safe) category by the FDA due to its excellent biodegradability, non-toxic antimicrobial activity, and biocompatibility. It has been known to exert bactericidal or bacteriostatic effects against a wide range of microorganisms[5].

The current study's objective was to evaluate the effects of esomeprazole, curcumin, chitosan, and of the combination of curcumin and chitosan on the expression of the vascular endothelial growth factor (VEGF) in stomach ulcers induced by ethanol in female rats.

2. Methodology

2.1. Experimental animals

We used 30 adult female rats with an average weight of 195.6 to 198.6 g. The rats were kept in specially designed cages, were given unlimited food and water. Before the experiments began, the rats we allowed around two weeks to get used to the new environment. Ethical approval was received for the herein described experiments (7-17-7922; November 3, 2022).

2.2. Drug and chemicals

Esomeprazole (manufactured by Ajanta Pharma Ltd, India) was used in this study. It was obtained from a local pharmacy in Hillah (Iraq); each tablet containing 40 mg of the drug. Curcumin (98%) and chitosan were purchased from Shanghai Macklin Biochemical Co Ltd (China). All other chemicals were bought from local commercial suppliers.

2.3. Curcumin–chitosan mixture preparation

After slowly adding 150 mg of chitosan (dissolved in 10 mL of 0.1-M acetic acid) to 40 mg of curcumin, the mixture was triturated in order to produce a uniformly yellow-coloured liquid[6].

2.4. Experimental design

Rats were randomly separated into six groups: two control groups and four treated groups (5 rats per group) as follows: (i) negative control group (distilled water; all rats in this group were given distilled water at a dose of 2 mL per rat, by oral gavage, during the experimental period), (ii) positive control group (ethanol; all rats in this group were given ethanol at a dose of 2 ml per rat, by oral gavage, in double dose after fasting for 19 h before the administration of each ethanol dose, (iii) esomeprazole-treated group (same as the positive control group, but after 1 h from the last ethanol dose, esomeprazole was administered in a single oral daily dose of 3.54 mg/kg and dissolved in 2 mL of distilled water, for 5 successive days), (iv) curcumin-treated group (same as the positive control group, but after 1 h from the last ethanol dose, curcumin was administered in a single oral daily dose of 40 mg/kg and dissolved in 2 mL of 0.06% (0.1 M) acetic acid, for 5 successive days), (v) chitosan-treated group (same as the positive control group, but after 1 h from the last ethanol dose, chitosan was administered in a single oral daily dose of 150 mg/kg and dissolved in 2 mL of distilled water, for 5 successive days), and (vi) chitosan–curcumin mixture-treated group (same as the positive control group, but after 1 h from the last ethanol dose, the chitosan–curcumin mixture was administered in a single oral daily dose of 2 mL, for 5 successive days).

2.5. Immunohistochemical staining

The sliced gastric tissues (5-μm thickness) were stained by using an Animal Research Kit in order to identify the VEGF protein expression. The standard proteins were obtained from MyBioSource (San Diego, CA, USA). The staining intensity for VEGF expression was evaluated by using scores from 0 to 3 as follows: 0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining. Data conversion to the numeric total immunoreactive score was established by using semi-quantitative anal-

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Table 1. Quantification of the intensity of the vascular endothelial growth factor (VEGF) staining and of the percentage of VEGF-stained cells in the gastric mucosa tissue of rats. Different letters indicate significant differences ($p<0.05$) among the studied experimental groups.

Experimental groups	**Staining intensity** (mean ± SE)	**Percentage of stained cells** (mean ± SE)	**Histochemical scoring** (mean ± SE)
Negative control group (distilled water)	2.750 ± 0.164 [a]	48.125 ± 3.399 [a,b]	135.625 ± 15.190 [a]
Positive control group (ethanol)	2.000 ± 0.258 [b]	41.313 ± 7.040 [a,b]	108.188 ± 22.595 [a]
Esomeprazole-treated group	2.583 ± 0.149 [a,b]	56.250 ± 6.885 [a]	156.250 ± 24.819 [a]
Curcumin-treated group	2.000 ± 0.162 [b]	36.250 ± 5.212 [a,b]	87.350 ± 16.526 [a]
Chitosan-treated group	2.208 ± 0.170 [a,b]	43.500 ± 7.288 [a,b]	122.833 ± 23.139 [a]
Chitosan–curcumin mixture-treated group	2.000 ± 0.204 [b]	32.063 ± 6.035 [b]	81.125 ± 20.121 [a]

ysis, in which the percentage of stained cells was multiplied by the staining intensity scores[7].

2.6. Statistical analysis

The data underwent statistical analysis with the use of SPSS version 23.0. The data are presented in the format of arithmetic mean ± standard error (SE). A statistical analysis was conducted by employing analysis of variance (ANOVA).

3. Results

The undertaken immunohistochemical staining of the rat gastric mucosa for VEGF showed positive staining in both control groups as well as in all treated groups (Table 1). However, the H-score for VEGF was found to show no significant change ($p>0.05$) in any of the studied groups.

4. Discussion

The present study reveals that the administration of esomeprazole, curcumin, chitosan, and of a mixture of chitosan and curcumin in rats with ethanol-induced gastric ulcers does not involve the gastric mucosal VEGF production. This result is not in agreement with previous studies that shown that VEGF expression is regarded as one of the main growth factors involved in the healing mechanisms of wounded tissues, through the stimulation of neovascularization processes[8,9]. In fact, the expression of VEGF is considered essential for the maintenance of gastrointestinal mucosal integrity, and angiogenesis is a pivotal mechanism contributing to the healing of gastrointestinal ulcers[10].

5. Conclusion

After attempting to characterize the effects of esomeprazole, curcumin, chitosan, as well as of a mixture of curcumin and chitosan on the expression of VEGF in gastric ulcers induced by ethanol in female rats, we have found no significant differences in terms of the H-score of this protein among the studied groups under the examined experimental conditions.

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Conflicts of interest

None exist.

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